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AN EXAMINATION OF THE CHROMOSOMES OF ANASA TRISTIS.

BY C. E. M'CLUNG AND EDITH PINNEY.

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I. INTRODUCTORY STATEMENT.

The extensive studies now in progress upon the chromosomes of numerous species of plants and animals have led to the formulation of several working hypotheses of considerable general importance. In order that these may be of value and serve as an aid to further progress, it is necessary that the phenomena from which they take their origin be extensively recognized and uniformly interpreted. Any conception of the relations existing between germ-cell organization, as exhibited by the chromosomes, and the structural characters of the body is now unfortunately delayed by certain disagreements

upon the actuality of the phenomena upon which such interpretations are based.

Fundamentally, these disagreements concern more or less intimately that view of chromosome organization which is called the "theory of chromosome individuality." Modern cytologists are either supporters or opponents of this theory, and its adequacy or inadequacy will be determined by the weight of evidence now accumulating. It is, of course, impossible to conceive that it will stand unmodified, for it is but the expression of our present limited knowledge; but it must now be determined whether these structures of the cell have persistent individualities or whether they are merely incidental and inconstant expressions of more fundamental phenomena. Observed facts must support one or the other of these alternatives, although in doing so they may readily enough modify the established order.

A considerable body of evidence has accumulated within recent years to support the theory of chromosome individuality and to associate the development of sexual characters with particular chromosomes. To this has been added the support of experimental work on sex determination and the parallelism between the segregation of Mendelian characters and the behavior of the chromosomes in the maturation divisions of the germ cells. Much of this cytological work has been done upon the insects, particularly in the orders Orthoptera and Hemiptera, and, in the large, there is a strong consensus of opinion. More recently confirmatory evidence has been furnished from studies upon nematodes, including Ascaris, by Boveri, upon echinoderms by Baltzer, and upon birds and man by Guyer. Here and there, however, there are workers who question the fundamental facts and deny the correctness of the theories founded upon them.

It is necessary, in order to proceed further with our interpretations, that we reach an agreement upon the observable facts in our science, and no honest question should remain without answer.

At present one of the most serious diversities of opinion exists regarding the character and the behavior of the accessory chromosome in the bug *Anasa tristis*. Because of the extensive and painstaking studies of hemipteran germ cells by Wilson, the contradictions and inaccuracies in the studies of

Paulmier and of Montgomery seemed to be cleared up and the phenomena in this order brought into agreement with those of the other insects. To this was added the confirmatory work of Stevens upon several orders of insects, the study of Lefevre and McGill on *Anasa*, and more recently that of Morgan on aphids and phylloxerans. Directly opposed to all this are the observations of Foot and Strobell upon *Anasa tristis*, the form studied in detail by Paulmier, Montgomery and Wilson. Their contention, in brief, is that the number of spermatogonial chromosomes is even instead of odd, that there is no dimorphism of the spermatozoa due to the presence in one-half their number of the accessory chromosome, and that there is no evidence to show that the theory of chromosome individuality receives any support from observed morphological continuity of any of the chromosomes. Aside from these major questions there are others, such as the history and fate of the plasmasome, upon which these authors are in disagreement with other investigators.

The issues here defined are so clear-cut and definite and their settlement of so much importance that the senior author of the present paper was led to offer his services for a reëxamination of the whole question. This offer met a hearty response from Professor Wilson, who sent not only live material, but his entire series of mounted slides, including those used by Paulmier. Professor Lefevre has been kind enough, also, to permit the use of his slides. Miss Strobell, writing for Miss Foot, expressed a lively interest in the proposed settlement of this problem, but was unable to send the preparations used in their studies, because these had all been destroyed on account of limited storage room. It is to be regretted that this material is not available for comparison, since it is upon it that the only divergent observations have been made; but since specimens from the same locality, prepared by all methods, including the special ones employed by the Misses Foot and Strobell, are at hand, there can be no reasonable doubt that it is representative.

To reduce the possibility of the influence of preconception to the lowest possible degree, the junior author has worked through the entire problem without knowledge of the work done by others, and without promptings from the senior author except such as were given by a series of questions for

which answers were desired. Independently, the senior author went over the same ground, first upon material prepared in this laboratory, and later upon the slides of Paulmier, Wilson and Lefevre. Whenever possible, photomicrographs were made, and representations of them accompany the paper. The two authors reached concordant results throughout, and their common experience is embodied in what follows.

Since the matters of dispute are purely of facts and interpretations, it will be necessary to take a definite position on each of the points involved, but so far as possible the material evidence for the decisions will be presented fully. It is hoped that this may be complete enough so that others interested may be able to form their own opinions directly from the appearances themselves. There will be presented first the personal work of the junior author, giving her independent results, and this will be followed by a general discussion of the problem by the senior author.

II. THE CHROMOSOMES DURING THE SPERMATOGENESIS OF ANASA TRISTIS.

BY EDITH PINNEY.

Preparatory to an attempted settlement of various disputed questions in the development of the germ cells of *Anasa tristis* an independent reëxamination of the entire process was undertaken. In order to avoid unconscious bias of opinion the previous reports on the spermatogenesis of this species were not read. Without referring, therefore, to the accounts of other observers for comparison or correlation it is purposed to record the results of these observations.

Material.—Preparation and general description.

Material was taken from specimens of *Anasa* collected in Lawrence, Kan., and in Woods Hole, Mass. The latter was obtained through the courtesy of Dr. E. B. Wilson, of Columbia University. All material was fixed in Flemming's fluid, the period of fixation varying from one-half to two hours. The longer fixation was found to give the better results. Paraffin sections were cut five micra in thickness and stained by Heidenhain's iron-hæmatoxylin, Flemming's tricolor and Auerbach's methods. Slides prepared by the author from nineteen individuals were studied. Most of the drawings were made at a magnification of 3625 diameters. This is reduced one-half in reproduction.

The testes of *Anasa tristis* are paired organs in the form of two small fig-shaped bodies which lie on either side of the median line in the cephalo-ventral part of the abdominal cavity. They are composed of a number of long, slender follicles lying parallel to each other and extending the entire length of the testis. The follicles themselves taper slightly from the broader base toward the sperm-duct. The follicular organization into cysts of germ cells of varying stages of maturation is similar to that in the Orthoptera, and the same method which was used to determine the sequence of the observed changes in the Orthopteran species is applicable here. Practically all of the cells of a cyst are in the same phase, although exceptions to this regularity are frequent in the older cysts, particularly those of the first and second spermatocyte generations. These variations are, however, to be expected, and may reasonably be accounted for if one considers the unavoidable differences which must occur in the rate of metabolism in cells situated differently in the same cyst.

The longitudinal section of a follicle presents some noticeable features of a structural nature caused by characteristic differences in the cells of various regions. The spermatogonial cells occupy the distal portion of the follicle, the extent of the spermatogonial area being determined by the age of the individual. These cells are small, and in the older cysts the nucleus is accompanied by little cytoplasm. Hence the peculiar staining quality of the nuclear material causes this region to appear more intensely stained than the proximal portion of the follicle. The cells, too, are crowded closely together. In contrast to this is the lightly stained spermatocyte region, due to the fact that here the cells contain a greater amount of cytoplasm in proportion to the amount of nuclear material. The cells have increased so greatly in size in the later generations that the cysts are larger. Usually a characteristic barrier exists between the two main regions of divisions. This is formed by cysts of cells undergoing the process which has been designated as synizesis, a detailed description of which will come later. In almost every instance, too, the line of separation is emphasized by the presence of many cysts of degenerating cells with their spherical masses of chromatin.

OBSERVATIONS ON THE SPERMATOGONIA.

From a study of polar views of the equatorial plate the number of spermatogonial chromosomes was determined to be twenty-one, and in no case was there observed a departure from this characteristic number. The chromosomes in young cells are larger than those in older cells and do not exhibit the tendency to coalesce so readily. Consequently cells for studying the number, form and behavior of the chromosomes were comparatively few, although enough were found to leave no doubt in the observer's mind as to the correctness of these observations and their interpretation. Figures 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, show polar views of the equatorial plate from spermatogonial cells. Certain elements of the complex exhibit constant and striking characteristics in size relationships. The largest three chromosomes in each group are easily identified, as are also the smallest two. The remaining members of the group more nearly approximate each other in size, so no attempt was made to homologize these in the several cells.

The form of the chromosomes varies from spherical and ellipsoidal to kidney-shaped with variations in size and position. The smallest two elements show the most striking differences in these respects from the other members of the group. There seems to be no fixed arrangement of the individual elements, except that there is an evident pairing of twin chromosomes. Sutton reported the presence of similar pairs in *Brachystola magna** and his observations have been verified by other workers in this laboratory on other species of the Orthoptera.† One element of such a pair is considered to be of maternal origin and the other of paternal origin. The tendency for equal elements to get together is very marked even in young cells, and makes conspicuous the presence of an unpaired element, one of the largest three chromosomes. Should its mate exist in the cell it could not possibly be overlooked when even the smallest elements are so prominent. Owing to uniformity in size, precisely which of the three is the unpaired element may be decided upon only when two of them are unmistakably paired. The smallest two elements show this tendency but rarely, their behavior at this stage being consistent with subsequent irregularities.

* "Spermatogonial divisions of *Brachystola magna*." Kansas University Quarterly, vol. 9, No. 2, 1900.

† The Chromosome complex of *Syrbula admirabilis*, W. R. B. Robertson. Kansas University Science Bulletin, vol. IV, No. 13, 1908.

All of the chromosomes lie with their long axis in the plane of the equatorial plate, and from a study of lateral views of this stage it is evident that the plane of the split in the chromosomes coincides with the same plane. Ideal conditions for equal division of chromatin are therefore present. Division is simultaneous throughout the group. The separated halves move toward opposite poles, maintaining their relative positions until the pole is nearly reached. Thus the shape of the spindle during anaphase is altered to accommodate the varying conditions. See figures 12, 13 and 14. There is no evidence as to the method of spindle-fiber attachment, and it is doubtful whether the matter can be decided in this material. Figure 15 is a drawing of a section through an entire cyst. Most of the cells are in metaphase. A few are in anaphase. The polarity exhibited in this section is typical of all cells of this generation.

In lateral views of anaphases in young cells the chromosomes are distinct. In the older, more crowded cysts the tendency of the individual elements to coalesce produces the appearance of a solid band of chromatin in the equatorial plate. Later stages of the same show two separate bands with spindle fibers between. The plate arrangement is lost when the pole is reached. Figure 17 shows a polar view of an early telophase. The chromosomes have not yet lost their identity, although they have apparently begun to disintegrate. Between the telophase and the succeeding metaphase there is an increase in the amount of chromatin. The formation of a nuclear membrane and the further dissolution of the chromosomes follow. A typical telophase from a young cyst is shown in figure 18. The daughter nuclei are flattened, but assume a spherical outline as the diffusion of chromatin proceeds. Figures 19 and 20 show the culmination of this process.

The inauguration of the next mitotic cycle is marked by the appearance of an exceedingly long and much coiled spireme, whether continuous or not it is impossible to say. At any rate, after the thread has thickened and shortened considerably it is plainly seen to exist in segments which finally condense to form the homogeneous bodies of the chromosomes. Figures 21 to 28, inclusive, show the successive steps in this transformation. The shortening segments show various twists, bends and contortions. See figures 22, 23, 24 and 25. Not until very late in the prophase does the longitudinal split in the threads become

visible. (Figs. 26 and 28.) At this stage pairing of equal elements is very common throughout the spermatogonial generations. Note the marked pairs in figures 1, 2 and 4, plate LXV. It would seem from this that throughout the history of the spermatogonial cells there is a consistent provision for the final processes which mark the transition from spermatogonia to spermatocyte. This process is called synapsis. The cells of a mature spermatogonial cyst have not been counted, but judging from the number of cells in a cyst of developing spermatids, eight mitoses complete the activity of the germ cells as spermatogonia, that is, that after the last division there are 256 cells in a cyst. In such cysts the cells are poor in cytoplasm.

After the final division the typical telophase is replaced by synizesis. During this process the chromatin is contracted into a deeply staining granular mass whose position within the nucleus is either central or slightly eccentric, leaving the peripheral area clear. It is quite certain that the chromatin mass is composed of intimately associated granular threads, for occasionally ends protrude from the knot, although entire threads can not be traced. (Figs. 30, 33.) It is true that the chromatin enters synizesis in the form of individual threads, and the presynizetic stage is characterized by the same elements. It is reasonable to suppose that the threads continue as such during the period of synizesis. That some change is taking place within the knot is shown later, but there is no evidence to show that this change involves the disorganization of the chromatin threads and its reorganization into new elements. Nothing can be said in regard to the duration of synizesis. That an end to end conjugation of the paired elements of the spermatogonial nucleus occurs during this period seems established beyond a doubt.

At one side of the conjugating threads lies a definite chromatin body of homogeneous consistency. Occasionally this body shows an uneven outline suggesting a slight disintegration of the constituent chromatin. Its position is constant and it is invariably present. From a study of subsequent maturation processes it is shown to be the accessory chromosome (McClung, Orthoptera) and can be identified as the homologue of the unpaired element appearing in the spermatogonial metaphases. In studying the history of this odd member of the complex, drawings of it in that stage were made, as well as

in numerous subsequent stages, for size comparisons. Fig. 14, plate LXVI, shows several drawings from different individuals. The spermatogonial cells from which drawings of the accessory were made vary in age, and as there is undoubtedly a decrease in the chromatin content of these nuclei as the cells multiply we would naturally expect to find homologous chromosomes varying in size in the same individual. The position of the chromosomes with respect to the equatorial plane varies previous to division, that is to say, in the formation of the equatorial plate the chromosomes may lie obliquely to the plane of the equator, and this would cause apparent differences in the size of camera drawings. Conceding that these possible causes of size variation are sufficient to account for the slight variations in size of the accessory as shown in the drawings, we are forced to conclude that the size of this element in the spermatogonia is strikingly constant. A similar study of the homogeneous body observed in the nucleus during synizesis shows a like constancy in size. Although these two elements which appear at different times in the nucleus are so unlike in form we cannot but consider them as equal chromatin masses. Moreover, if synapsis is a conjugation of twin chromosomes, obviously the unpaired element of the earlier stages would take no part in this process. This evidence is sufficient to incline us to believe that the two masses are identical. Further evidence will be given to strengthen this conclusion. An account of the presynizetic stages or growth period will be given in the observations on the first spermatocyte generation.

The First Spermatocyte.

The earliest condition of the spermatocyte cell is the result of the disentanglement of the chromatin knot formed during synizesis. Even before the chromatin has spread to the nuclear membrane, and while the peripheral area is comparatively free from it, it is easy to determine that the chromatin exists in the form of granular threads or rods. See figures 1, 2, 3 and 4, plate LXVI. This diffusion of the chromatin threads makes possible the detection of a second body similar in size and staining qualities to the chromatin body described as present during synizesis. This body is not present before synizesis, is not apparent during the synizetic period, and is present afterward in the interior of the nucleus. The natural conclusion then is that this is a product of synizesis. Its staining qual-

ities indicate that it is composed of chromatin. The distinction between this and the accessory is only possible when the accessory lies close to the nuclear membrane, which is its usual position. In this respect it resembles the accessory chromosome of the first spermatocyte prophase of the Orthoptera.

The history of the three distinct nuclear components, active chromatin, accessory chromosome and plasmasome, will be followed separately up to the spermatocyte metaphase, beginning with the chromatin. The reorganization of the nuclear chromatin into typical spermatocyte chromosomes after diffusion is completed gives no hint of a reversal of the mitotic cycle. That is, the appearances which characterize chromosome reconstruction do not suggest a reversal of the changes which were observed to take place in the diffusing chromatin. The straight threads which typify the early prophase (figs. 1-4, plate LXVI) gradually elongate and finally are disposed into a fine reticulum at the intersections of which are very slight accumulations of chromatin. See figure 4, plate LXVI, and figure 4, plate LXVII. This might be considered as the climax of diffusion, for in this stage the chromatin is most evenly distributed throughout the nucleus. Subsequently there are more extensive accumulations of chromatin at certain points within the nucleus. These amorphous aggregations of granular chromatin gradually assume the form of loosely organized tetrads resembling a few of the types of tetrads found in the Orthoptera. Figure 16, plate LXVI, shows four types of tetrads taken from one cyst all of the cells of which are in the same stage of development; (*a*) shows the largest form. By analysis it is seen to be composed of four equal chromatids and represents the pair of the largest spermatogonial chromosomes conjugated. The disposition of maternal and paternal chromatids is indeterminable in this as well as in the forms shown by (*b*) and (*c*), which are different types of the cross. In each case two opposite arms of the cross are composed of portions of maternal and paternal threads lying parallel to each other. The ends of these arms are the synaptic ends of the chromatids, but whether the synaptic end of a chromatid represents the polar end, as is certainly the case in the Orthoptera,* it is impossible to determine. One of the other pair of opposite arms is composed of parallel maternal threads and the remaining arm consists of parallel paternal threads;

* Pinney, Edith, Organization of the Chromosomes in Phrynotettix magnus, Kansas University Science Bulletin, vol. IV, No. 14, 1908.

(*a*) shows a rod form in which the division at right angles to the long axis may or may not separate elements of opposite parental origin. It is impossible to ascertain whether these rod forms have passed through the cross stage, since some of the earliest recognizable tetrads have this form and keep it as they condense.

The manner in which these forms are evolved from two conjugated chromosomes of opposite sex, each split longitudinally, is similar to that described for the Orthoptera by McClung;* (*d*) is an unusual condition at this stage of the smallest spermatogonial chromosomes. Even in stages as late as those shown in figures 22 and 24, plate LXVI, the separate halves of this pair often lie on opposite sides of the nucleus. Just previous to the metaphase they approach end to end. The constriction between the two halves is never obliterated by a close union. Evidently synapsis has had no effect on the smallest two members of the spermatogonial complex, although they undoubtedly entered into the synaptic knot. Subsequent changes consist in the condensation of these filiform tetrads to form the homogeneous elements of the metaphase. As the chromosomes approximate the homogeneous condition they arrange themselves at the periphery of the nucleus. This tendency may account for the characteristic position of the accessory during synizesis, the growth period and the spermatocyte prophases. The same nuclear membrane which is formed previous to synizesis continues to function up to the time of a late spermatocyte prophase. At the latter stage there are present within the nucleus thirteen distinct bodies. Figures 10 and 11, plate LXVI, show two parts of the same nucleus appearing in adjacent sections. The thirteen bodies are drawn. These must represent the twenty-one spermatogonial chromosomes plus the plasmasome created during synizesis.

Before stating the homologies between these spermatocyte structures and those of the previous generation the history of the accessory will be followed, beginning with synapsis. In this connection the behavior of the new spermatocyte element, the plasmasome, will be reported. The time of appearance, the nature and source of these two bodies, have already been spoken of. It has also been mentioned that immediately after synizesis the two bodies are practically equal in size. In the succeeding

* McClung, C. E., '00, The Spermatocyte Divisions of the Acrididæ, Kansas University Science Bulletin, IX, 1, '02; The Spermatocyte Divisions of the Locustidæ, ibid., XI, 8.

diffusion stages the plasmasome increases in size and the distinction between it and the accessory becomes very marked. In these stages the accessory remains closely appressed to the nuclear wall while the plasmasome is located internally. See figures 3, 5 and 6, plate LXVI. This period during the diffusion of chromatin and the growth of the plasmasome can not be considered as belonging to the prophase of the first spermatocyte. The nucleus increases steadily in size, reaching its maximum when diffusion is complete, so we may consistently call this interval after synizesis the growth period. Its culmination marks the installation of important changes, one of which, the re-formation of the chromosomes, has already been described. The accessory meanwhile maintains its former position, size and staining qualities. During the prophase the plasmasome gradually diminishes in size, and finally disappears just before the metaphase. During this time, too, it does not stain so readily with chromatin stains. In the Heidenhain's iron-hæmatoxylin preparations the staining quality of the plasmasome varied with the amount of extraction in the iron-alum solution. Occasionally the plasmasome presents a vacuolated appearance. This may occur at any time during its history but is more characteristic of the beginning of its dissolution. Occasionally in the prophase there occur two small homogeneous bodies staining like the plasmasome. As the typical plasmasome is absent and the accessory present these are considered as fragments of the plasmasome. There is no regularity in their occurrence. The cysts in this condition are not frequent and the majority of their cells contain typical plasmasomes.

In later stages the plasmasome is identified by its shape, becoming spherical as it decreases in size. Figure 7, plate LXVI, shows a nucleus containing the plasmasome and the accessory. The difference in staining qualities is apparent. The accessory remains in its former position, its form to the observer depending on his point of view. Plate LXVII consists of a series of drawings showing the size relations of the accessory and the plasmasome at the time of synizesis, the growth period and the succeeding prophases. The drawing of nuclei at the top of the page show the condition of the active chromatin. They are numbered in the order of the changes which they present. For instance, figure 1 shows a nucleus during synizesis. The perpendicular column below figure 1, designated 1*a*, shows a

number of drawings of the accessory taken from cells in the same stage as figure 1. Figure 2 shows a nucleus of the stage immediately following synizesis. The perpendicular column, 2*a*, below figure 2 shows a number of drawings of the accessory taken from nuclei in the same stage as figure 2. The perpendicular column, 2*b*, beneath figure 2 shows a number of drawings of the plasmasome taken from cells in the same stage as that shown in figure 2. In the same way columns 3*a* and 3*b*, 4*a* and 4*b*, etc., are drawings of the accessory and plasmasome from nuclei such as are shown in figures 3, 4, etc., respectively. The horizontal columns *A*, *B*, *C* and *D* indicate the different individuals from which the drawings were made. The differences in size which are shown in these monoplane drawings depend upon the point of view of the observer. That the accessory is constant in size is easily determined by different focusings. From these drawings there is also found to be a regularity in the relation between the size of the plasmasome and the condition of the active chromatin of the nucleus. From the foregoing observations it is now clear that the thirteen nuclear structures present in the late spermatocyte prophase comprise one accessory, one diminished plasmasome, two small chromosomal elements of opposite parental origin, and nine ordinary tetrads.

Figures 1 to 10, inclusive, plate LXVIII, show typical polar views of the first spermatocyte metaphase. All of the groups shown are from different individuals, with the exception of figures 2, 3, 4, plate LXVIII, which were in the same section of the same cyst. As many as six of these views, all typical, have been observed in one section in one cyst. There are eleven chromosomes present, each of which, with the exception of the accessory, is composed of four chromatids. The plasmasome has disappeared, also the nuclear membrane. The chromosomes lie in a clear area surrounded by the cytoplasm. Nine of the complex, including the largest, form a rather compact ring, in the center of which appears the smallest member of the group. The relative positions of the chromosomes forming the ring vary. One chromosome lies outside of the ring. Figure 11, plate LXVIII, shows six of these outer chromosomes at this stage taken from the same individual from which figure 6 was made. So, also, figures 12, 13, 14 and 15 are drawings of the similarly located chromosome found in the individuals from which figures 7, 8, 9 and 10, respectively, were made. This

eccentric member shows remarkable constancy in size. Its size and its position suggest the accessory during synapsis. See figures 29, 30, 33, plate LXV. Figure 5, plate LXVIII, shows a polar view of an atypical metaphase. This condition was observed only twice in this material, both times occurring in different individuals. There appear to be twelve chromosomes present. In addition to the small chromosome within the ring, there are two large equal elements which are not large enough to correspond to any elements present in the typical metaphase. Only eight chromosomes form the ring. It must be that the missing one of the nine which usually form the ring is represented by the two small and unusual bodies which lie within the ring, one on either side of the small element, and that this is merely an accidental case in which synapsis did not occur. In connection with this supposition we recall the fact that synapsis did not occur in the small chromosome at the usual time. This similarity of action during the synaptic period may be significantly related to their similarity of position.

In a lateral view of a spermatocyte anaphase the same surface of the chromosomal elements is presented to the observer as is seen in the equatorial plate of the spermatogonial cells. By referring to figures 1 to 10, plate LXV, it will be seen that the chromatids have become slightly shorter and correspondingly thicker. Here again the smallest chromosome asserts its independence. In the spermatogonial cells these small chromosomes present spherical outlines. In the first spermatocyte generation, instead of shortening, as their fellows, they have elongated. Only in this element and in the accessory was the plane of division actually determined. Since the two halves of the smallest chromosome are never so closely united that we lose sight of their identity, it can be stated positively that division in this case is transverse and qualitative, causing the separation of maternal and paternal elements. Owing probably to the slight attraction exhibited between the halves of this chromosome division is effected while the other chromosomes are still intact. Lateral views of early anaphases invariably show this condition. Similar views of later stages show its separated halves preceding the larger diads to the poles. In the ordinary tetrads the formation of the crosses might be interpreted to have the same significance as is attributed to the like form in the Orthoptera, in which McClung

has shown conclusively that the first division plane is longitudinal. However, in this case there is no positive evidence to offer. The chromosomes enter the equatorial plate with their longitudinal axes parallel to the spindle fibers. Figures 16 and 17, plate LXVIII, show that the polar ends of the chromosomes converge toward the poles of the spindle. That both methods of division may be employed by different members of the group is shown by a study of the accessory at this stage. This element, which is composed of two chromatids, was last observed in the metaphase lying outside of the ring. The element (x) in figure 16, plate LXVIII, is undoubtedly the accessory chromosome. This drawing is not limited to one plane. The small chromosome is shown dividing in its usual manner. This element, it will be remembered, is located at the center of the ring and no element of the ring could lie so far from this center as the one marked (x). This, then, must lie on the outside of the circle. From the arrangement found in polar views of the metaphase one would expect in the lateral views to find sections which would show four chromosomes in the same plane. These would include the small central member, two chromosomes of the ring, one on either side, and, at one side, the accessory. Such a section is shown in figure 17, plate LXVIII. The small chromosome, it will be seen, occupies the axis of the spindle, which in this view appears asymmetrical, the asymmetry being due to the extra divergence of the fibers attached to the accessory.

In these lateral views of anaphases the structure of the accessory is easily determined. It is composed of two chromatids, which are shown in figures 19, 20 and 21, plate LXVIII. All of the other chromosomes contain four chromatids, although in some only two of the four appear in the same plane. The accessory is easily identified by its form in these stages, where it is shown to divide tardily, division not beginning until the ordinary chromosomes have entirely divided. In their journey to the poles the accessory chromatids lag behind the others. Figures 17 to 26, plate LXVIII, show this behavior at different stages. All of the chromosomes of the complex keep their relative positions until the pole is reached. Figures 1, 2, 3 and 9, plate LXIX, show polar views of the dividing groups with the inner and outer members in their characteristic positions. As soon as the pole is reached the

chromosomes crowd together and, on account of the lagging of the accessory, lateral views of this stage show the condition reproduced in figures 25 and 26, plate LXVIII; (x) is the accessory. It is easily identified in both daughter groups. Figure 24 shows the only case observed which might be interpreted as the failure of the accessory to divide. The spherical form of the structure (x) may indicate a degenerate form of the accessory or an unusually persistent plasmasome. The crowding together of the diads at the poles completes the cycle of changes occurring during the first spermatocyte generation. No telophase involving the disintegration of the chromosomes and the appearance of a new nuclear membrane occurs.

The Second Spermatocyte.

The second spermatocyte generation begins with the formation of an equatorial plate by the members of the new spermatocyte nucleus. These second spermatocyte chromosomes consist of ten diads and one monad or single chromatid, the accessory. In figures 1, 2, plate LXIX, of typical equatorial plates of this generation will be seen a tendency toward the same chromosome arrangement which prevailed during the preceding mitotic cycle. From the size relations shown in figure 4, plate LXIX, which is a group of accessory chromosomes from cells of the same stage and animal as figure 3, plate LXIX, respectively, we are justified in our conclusions as to the identity of this element. Figures 5, 6 and 7, plate LXIX, are polar views of beginning anaphases. The chromosomes are viewed here in cross-section. Figures 10, 11 and 12, plate LXIX, show lateral views of the beginning of this ultimate division. Figure 10 suggests what transpired between the stage shown in figures 1, 2 and 3 and that shown in figures 5, 6 and 7. The transition is extremely brief, for evidence of it is comparatively infrequent. The chromatids which, during the first spermatocyte division, lay parallel to each other have separated at one extremity and have swung around until they lie end to end, the separate ends pointing toward opposite poles. The chromatids maintain this position during a relatively long period of time, for this condition and the following telophase are most commonly noted in cysts of second spermatocytes. A typical spindle is formed and the fibers are attached at the separated ends of the chromatids. Separation of chromatids occurs simultaneously, the smallest member here acting in unison

with the others. This may be regarded as due to the fact that the same conditions are present in all of the chromosomes previous to division; that is, the degree of union between the chromatids is the same in each diad, while it will be remembered that this relation between the diads which composed the tetrads of the first spermatocyte varied.

When the chromosomes separate sufficiently to expose a clear zone of fibers in the region occupied by the equatorial plate we find the accessory chromatid still in its place, and it keeps this position until the remaining chromatids have almost reached the opposite poles. Figures 13 to 18, inclusive, plate LXIX, show the successive steps in this division. Well coalesced daughter groups of this division are observed at opposite ends of the spindle, while the accessory lingers half way between. The accessory finally approaches one of the groups and almost immediately the dividing cell wall is formed. This leaves the accessory near the newly formed cell wall, entirely apart from the group to which it evidently belongs. Figure 19, plate LXIX, is a group of such bodies drawn from cells in this stage. The size of this lagging body was thus found to be constant. The spindle fibers between the two groups persist until after the nuclear membranes of the daughter cells are formed. Before this occurs, however, the changes shown in figures 20 to 25, inclusive, plate LXIX, take place. This consists in the withdrawal of the accessory from its position near the dividing wall into the now shapeless chromatin mass of the new nucleus. In studying these processes it was very easy to obtain sections which showed the two daughter cells in the same plane. The connecting band of spindle fibers leaves no doubt as to their relation even after the new cell wall has been formed. The accessory occurs in only one of these cells. A further significant fact is that in every such case where both daughter cells can be identified beyond doubt one always shows the accessory at some stage of its characteristic activity, while in the other no corresponding body is to be found.

As soon as the membrane forms around the spermatid nucleus which contains the accessory this element becomes detached from the mass of which it forms a part and moves again to the nuclear wall. Its individuality again becomes prominent. Figures 26 to 31, plate LXIX, show this condition. Here also the same precautions in determining its identity were observed.

Care was taken to study both daughter groups, in one of which it invariably occurred while the other offered no evidence of the presence of a similar element. The constancy in size of the accessory and the relation of its size here to the size of the accessory as determined by figures 4 and 19, plate LXIX, help to establish its identity. This condition of the chromatin in the spermatid nucleus lasts for only a short time. The large chromatin mass soon begins to break up into smaller masses which eventually distribute themselves against the nuclear wall. In an early stage of this disintegration the accessory is still easily recognized, as it lies on one side of the nucleus apart from the rest of the chromatin. It still keeps the elongated form it displayed in the second spermatocyte anaphase.

A whole cyst of young spermatid nuclei was counted in order to determine in just what proportion of the spermatids of one cyst the accessory occurred. The importance of this observation is obvious, although it seems that the result could be predicted safely from the evidence obtained from previous observations. An account of the method used in counting may help to verify the results. Serial sections of an entire cyst were obtained. The cyst chosen for counting ran through twenty-four sections. The sections were five micra in thickness. The exact condition of the chromatin was found to vary in almost every cyst of this generation. In the cyst counted were found the stages shown in figures 28, 29 and 31, plate LXIX. From the conditions existing in such nuclei we would expect to be able to identify the accessory from its shape and its position with respect to the rest of the chromatin in the nucleus. Evidently the number of nuclei in such a cyst and the number of spermatids formed would be equal. All whole nuclei or almost whole nuclei and fragments containing the accessory were counted. It was impossible to avoid the error of counting one nucleus as two where a nucleus happened to be cut exactly in half; one half appearing in two adjacent sections. The number of nuclei containing the accessory was 484. The number of fragments of nuclei which contained the accessory was 40. Thus the entire number of spermatids in which the accessory occurred was 524. The number of nuclei counted which contained no accessory was 577. These, however, would include the nuclei from which small fragments containing the accessory had been severed.

That would make the number of nuclei with no accessory 537, which is slightly larger than the number with the accessory. It has been pointed out that the nuclei which were cut in two equal parts might be counted as two. This would add to the number of cells which did not contain an accessory. Considering this source of error the number of cells of each kind may practically be considered as equal. There can be no doubt as to the occurrence of two kinds of spermatids which develop into spermatozoa, one containing one more chromatid, the accessory, than the other; and from the evidence presented we feel justified in concluding that the two kinds of spermatozoa occur in equal numbers.

III. A COMPARISON OF THE RESULTS OF STUDIES UPON CHROMOSOMES OF ANASA.

BY C. E. M'CLUNG.

1. *Methods.*

The methods of an investigator are a very important factor in arriving at conclusions, and it is quite possible to secure diametrically opposite results upon the same material by varying the technical processes. It will, therefore, be necessary to consider the methods of preparation and study employed upon *Anasa*. Paulmier, Montgomery, Wilson, Lefevre, McGill and Pinney fixed their material with Flemming's fluid, corrosive-acetic mixtures, Bouin's fluid and various other cytological reagents. This material was then sectioned in paraffin, and stained, principally with the iron alum—hæmatoxylin stain of Heidenhain. The slides thus prepared by Paulmier, Wilson, Lefevre and Pinney are beautiful examples of cytological technic and leave little to be desired in delicacy and precision. A considerable number of the slides sent me by Professor Wilson and Professor Lefevre are prepared according to the methods of Foot and Strobell, so that I have been able to study the material under their conditions, although, unfortunately, their own preparations have not been accessible to me. Since the entire case of these investigators, as opposed to all their fellow workers, rests upon their own peculiar methods, it seems desirable to consider in detail the technic upon which they rely.

The usual methods of cytologists are dismissed with practically no consideration, and instead of fixing the material, it

is spread upon glass slips and allowed to dry. (Foot and Strobell, '07, p. 282.) In this condition it is stained with Bismark brown and mounted in balsam. Entire reliance is placed in this method, and little attempt is made to correlate the results thus obtained with the appearances presented by other systems of technic. That the position of these authors on this point may be clearly understood, I quote their statement of the case.

"Professor Wilson, in his recent paper in *Science*, February, '07, replying to our preliminary note, says that he thinks the contradiction in our results is probably due to the difference of method employed, we having placed our faith in smear preparations, while he has relied on sections. We are glad of an opportunity to emphasize this faith, believing that for demonstration of the structure and count of chromosomes our modified smear preparations are more reliable than sections; and it is for this reason we have abandoned the use of sections in studying chromosomes, except for comparative work and for studying the topographical relation of the cells. In cells fixed and sectioned nearly all the delicate details shown in the chromosomes of our smear preparations are completely lost, and it ought to be too obvious to mention that a method which presents clearly each individual chromosome in its integrity offers decided advantages when the question of accurate counting assumes the importance and develops the contradictions familiar in recent literature."

The authors state that they have used sections for comparison and topographic work, but none of their photographs are made from sections, and their entire argument, as published, is based upon material prepared according to their own method.

It is my judgment that this method, *used alone*, is entirely inadequate for accurate results, and in this particular case is responsible for the discrepancy between these investigators and Paulmier, Wilson and Montgomery. From the work of Miss Pinney it will be clear, I think, that in the prophases figured by Foot and Strobell there are two darkly staining bodies, the accessory chromosome and the plasmasome, instead of the one shown in their photomicrographs. Owing to the technic employed by them the plasmasome is practically destroyed, and instead of using other methods to determine the

nature of the nuclear body, they *assume* that it is a plasmasome. "As the presence of a plasmasome in the nucleus at these stages is the typical phenomenon familiar in all known forms (its absence being most exceptional), we feel justified in interpreting the structure we find in the resting nucleus as a true plasmasome, and not an odd persisting spermatogonial chromosome, *i. e.*, chromosome nucleolus." (Foot and Strobell, '07, p. 284.) Further, the smear method does not give precise and accurate pictures of the chromosomes. The variation in size, owing to the different thicknesses of the smear and amount of spreading, is very extensive, as may be seen by a comparison of the photomicrographs of Foot and Strobell. Compare, also, figures 21-26, plate LXX, which represent smears of cells in the same stage. Not only may the entire groups of chromosomes in similar cells vary as three or four to one, but the members of one cell may suffer unlike expansion or contraction to a similar extent, if the conditions of drying are variable, due to the thickness of the smear film. There is but one advantage of the smear method over sections, and that is that it presents all the elements of the cell in one plane, so that their entire outline is visible for studying and photographing. It is a method that should be used in connection with sections, and from the beginning of my work I have utilized it consistently and with great profit in this way. I therefore speak of it as a warm friend and advocate, but at the same time I realize its limitations and am convinced that it should not be used to the exclusion of all other methods.

Foot and Strobell have employed photography alone as a means of presenting illustrations of their material, and it is assumed by them that if a thing can be photographed it must necessarily be a true picture of normal conditions. This I consider to be a decided fallacy. A photograph is an interpretation by the observer, just as is a drawing. The personal factor is no more absent from one method of illustration than it is from the other. Photographs may present with greater fidelity the details of structure in an object, but the choice of the object and the nature of details are at the command of the photographer. It is possible, especially in smear preparations, to select cells that will illustrate almost any condition and to photograph them. The highly interpretative character of the photograph is illustrated admirably by the present con-

troversy over the chromosomes of *Anasa*. Foot and Strobell publish photographs that seem to show that the accessory chromosome divides in the second spermatocyte division. This is their interpretation of the behavior of this element, and they illustrate it by photography. Not a picture which they publish would indicate that there is ever a case where the accessory chromosome positively fails to divide at this time, and yet literally thousands of unquestionable cases of this occur in every good preparation. See the group of cells shown in figure 15, plate LXXI. By selecting a series of early anaphases, and a very few obscure and distorted individual cells, they are able to secure pictures which appear to bear out their contention. In speaking thus of the methods of Misses Foot and Strobell, I wish it clearly to be understood that I am not reflecting upon their motives. It is my desire only to show that photographs are interpretations of observed phenomena and have no claim to infallibility. Again, as in the case of the smear method, I would state that I speak as a friend of the method and not as one who deprecates it. I believe that scientific papers, particularly cytological, should, whenever possible, be illustrated by photographs, and in my own publications I have followed this plan so far as the circumstances would permit.

2. *What is the number of spermatogonial chromosomes and of oögonial chromosomes?*

Confessedly, the accurate estimation of the numbers of chromosomes in these generations of cells is a difficult matter, and if it were the only means of determining the chromosomal differences of the sexes there might be occasion for doubt with regard to theories founded upon such enumerations alone. Fortunately, it is only one of several criteria of sexual differentiation in the germ cells, and so is not of commanding importance. Accurate counts of the chromosomes, however, are entirely possible with care, and concordant results are obtainable by independent observers. In the case of *Anasa* this is quite possible, and both Miss Pinney and myself have found the number twenty-one uniformly present in the spermatogonia. Not a single instance of twenty-two chromosomes was found in any spermatogonium, and we must therefore conclude with Wilson that thirty-one is the normal spermatogonial

number. In plate LXX, figures 1-3, may be seen spermatogonial complexes showing this number clearly.

It is claimed by Foot and Strobell that the number is twenty-two, and they publish four photographs in support of this view. Only one of these represents a stage where the chromosomes are compact and homogeneous, and this shows twenty-one chromosomes, although in the explanation of the figure the authors state that one of the apparent chromosomes is really two joined at right angles. Since there is no case in the figure where one chromosome is bent at almost right angles, the explanation is not very convincing. The remaining three are of earlier stages, in which the chromosomes have a loose structure that might readily be disturbed in the process of smearing, thus separating one of the larger chromosomes. In two of the photographs such an interpretation would seem to be entirely justified. Photo 49 is the only one in which there would seem to be any certainty with regard to the number twenty-two. Here it appears as if conditions were normal, and it might be recorded as an instance of twenty-two chromosomes in the spermatogonia.

The conditions regarding the spermatogonial number may be summarized thus: Paulmier, Montgomery at the time agreeing with him, reported twenty-two spermatogonial chromosomes. Later, Wilson, working upon Paulmier's original preparations in part, finds twenty-one spermatogonial chromosomes, and Montgomery, restudying his own material, agrees with this enumeration. Lefevre and McGill report twenty-one spermatogonial chromosomes from their own preparations. Foot and Strobell, working upon smear preparations, consider twenty-two to be the normal spermatogonial number. Miss Pinney, upon examining material from Kansas, Massachusetts and Pennsylvania, and working without a knowledge of others' results, finds twenty-one to be the spermatogonial number. Finally, from a study of the original material of Paulmier, Wilson, Lefevre and McGill, and Pinney, I myself find in all clear cases, where there is no doubt of the presence of all the chromosomes, that the number is twenty-one. Foot and Strobell object to the testimony of Montgomery, and of Lefevre and McGill, on the ground that they reported at one time an even number of spermatogonial chromosomes and at another an odd number. It must of course be admitted that this weakens their

testimony, for it is an evidence either of careless work or of the influence of preconceptions. I can recall with what difficulty I persuaded myself, against the current conceptions of chromosome numbers, that the spermatogonial number of chromosomes is odd in the Orthoptera, and I can easily understand the temptation, in an early stage of an investigation, to seek concordance with accepted opinions. The investigators who reported even numbers for their material did so when all the evidence from other forms seemed to demonstrate the universality of this phenomenon. In a sense their testimony is more valuable after a restudy of their material, because it indicates the desire to arrive at facts regardless of consequences. It is a very unusual man, or a very stubborn one, who finds no occasion to change his opinions.

This is the present status of the question concerning the spermatogonial number of chromosomes in *Anasa*. If it is impossible for the interested cytologist to come to a conclusion regarding the facts of the case from the studies so far made, he is quite at liberty to undertake an investigation for himself and I have no doubt but that all the material that has been available for my investigation will be placed at his disposal.

There appears to be no dispute regarding the oögonial number, although the counts in these cells have been less numerous than in those of the male. So far as the opportunity has offered I have gone over the female complex of chromosomes, and I have no reason to doubt that the number is twenty-two, as determined by Wilson.

3. *What is the number of first spermatocyte chromosomes?*

Regarding the number of first spermatocyte chromosomes, there seems to be no difference of opinion, since every observer has recorded eleven as typical for this generation. The clearness with which the chromosomes of the first maturation mitosis appear in the equatorial plate almost precludes the possibility of error. Here also may be found a close adherence to a type of arrangement which presents the small chromosome in the center, surrounded by a more or less regular ring of nine chromosomes upon the outside of which is placed the accessory chromosome. This is not an invariable arrangement, for it is a culmination of the movements of the chromosomes in the prophase where the elements bear somewhat similar relations to each other, but it appears in a large proportion of the cells.

The position of the accessory chromosome upon the outside of the group has led Foot and Strobell to coin another new name for it, and in their terminology it is the "eccentric chromosome."

4. *What is the behavior of the accessory chromosome and of the plasmasome in the first spermatocyte prophase?*

While there is no dispute regarding the number of chromosomes in the first spermatocyte, there is serious divergence of opinion with reference to the structure of the accessory chromosome. Upon its nature depends the number of spermatogonial chromosomes and the character of the four spermatids derived from each first spermatocyte, so its determination is most important. If it be a tetrad then the spermatogonial number must be even and the spermatids all alike in the possession of eleven chromatids; but if, on the contrary, it be a diad the spermatogonial number of chromosomes is necessarily odd and the spermatids of two types, one of which possesses eleven chromosomes and the other ten.

With the exception of Foot and Strobell all observers are agreed that there are but two chromatids in the accessory chromosome, but these observers claim that it possesses four as do the other chromosomes. As proof for this contention, they publish a series of chromosome groups from the late prophase in which the various elements are clearly shown. The identification of the accessory chromosome is correctly made in each case, I believe, but it is entirely clear to my mind that there is nothing in the appearance of these photographs to justify the interpretation of the accessory chromosome as a tetrad. There is in each case a characteristic difference between the tetrads and the accessory chromosome, for in the former there always appears a diamond-shaped opening, the angles of which point to the two lines of cleavage, while in the case of the accessory chromosome this is lacking. Every accessory chromosome, on the contrary, shows a clear, straight line of division along its entire length. In but a single instance is there an exception, and that is in their figure 4, plate II, where the plane of longitudinal cleavage is interrupted by transverse markings. A careful examination of this chromosome will demonstrate, however, that there is not only *one* of these apparent cross divisions, but *two* of them. I am convinced that the excellent photomicrographs of Foot and Stro-

bell of this stage are sufficiently clear in their evidence of the nature of the accessory chromosome, and I have not included any number of these stages in the photomicrographs accompanying this paper. Moreover, in none of the material that I have examined is there any indication whatever of a tetrad structure in the accessory chromosome, and I am thoroughly convinced that it is a univalent chromosome.

If the earlier prophase of the first spermatocyte be examined, the chief error of Foot and Strobell becomes evident. It is claimed by these investigators that the accessory chromosome behaves during the prophase as do the other chromosomes and that there is but a single nucleolus-like body present —a plasmasome. The work of Miss Pinney showing the transition in form and size of the accessory chromosome and of the plasmasome, both of which appear simultaneously in the nucleus, makes it entirely clear that Foot and Strobell were mistaken on this point. As I have elsewhere pointed out, the technic of these investigators is responsible for their failure to find the plasmasome, for it is this structure, and not the accessry chromosome, that is missing from the smear preparations.

These investigators have pointed out the large difference between the appearance of the accessory chromosome in drawings made by Wilson and the photographs illustrating their own paper. Such variations in the form of this element will receive explanation in part from the series of drawings made by Miss Pinney to illustrate the transition in form of the prophase elements. Both the stage of development and the method of preparation influence the form of this accessory chromosome. As a rule, sections show this element as a spheroidal, apparently homogeneous body during most of the prophase, but occasionally, just succeeding synizesis, it appears as a short, thick thread, with a tendency to be bent at the middle. This is the nearest approach to a spireme condition that it attains, and in this corresponds to similar conditions which I have described for certain Locustids. So far as my experience goes it would seem to be the rule that the accessory chromosome, to a certain degree, undergoes a granular diffusion of the chromatin similar to that of the other chromosomes, but that it is less extensive and is frequently marked by the close approximation of divisions of the thread.

It will be observed from plate LXX, figures 4-6 and 11, that

the accessory chromosome in this spireme condition is accompanied by a large distinct plasmasome which lies toward the center of the nucleus. Such a structure is absent from the photographs presented by Foot and Strobell when the accessory chromosome (their plasmasome) is elongated. As will be noted in Miss Pinney's description, the plasmasome does not appear until after synizesis is established. Some of the nuclei figured by Foot and Strobell would seem to be presynizetic in development and could not therefore contain plasmasomes.

I would conclude, therefore, that after synizesis there are present in the first spermatocyte nuclei two nucleolus-like bodies, one the accessory chromosome and the other a plasmasome. Further, that the accessory chromosome exists throughout the whole period, for a time, as a short, heavy thread, at other times concentrated into a mass, and finally in the late prophases as a straight longitudinally split rod. The plasmasome, on the other hand, is an inconstant structure, absent before synizesis, and again in the late prophase, and is not to be mistaken for the accessory chromosome.

5. *What is the behavior of the accessory chromosome in the first spermatocyte mitosis?*

Reference has already been made to the condition of the accessory chromosome in the late prophase and to its position in the equatorial plate. It appears in the metaphase as a univalent, longitudinally split rod lying outside the ring of ordinary chromosomes. In division its halves separate and during the anaphase move to the two poles of the spindle as do those of the other chromosomes. Examples of this may be seen in figures 2, 3, 4, 5, 6, 7, 8, 9, 10, plate LXXI. Both series of daughter chromosomes may be seen in plate LXXI, figure 3, which shows a mid-anaphase with the halves of the accessory chomosome accompanying each group. There appears to be no question on the part of anyone regarding the movements of the chromosomes in the first spermatocyte, so that an extended discussion on this point is not called for.

From the fact, however, that Foot and Strobell have used the tardy division of the accessory chromosome in the first spermatocyte as an argument to prove its division in the second spermatocyte, because here also it lingers for some time near the equatorial plate after the other chromosomes have moved toward the poles of the spindle, it will be necessary to call at-

tention to figures 6, 7, 8, 9, 10, plate LXXI. These show that while the time of division is later than that of the other chromosomes the actual separation occurs and the halves of the divided accessory chromosome may, in each case, be seen in the daughter cells. No instance could be observed where there could be doubt of this fact. The clearness of these phenomena is due to the fact that the accessory chromosome remains, as in the prophase, distinct from the other chromosomes, so that it can be followed even into the telophase. As will be seen later, the same aloofness on the part of the accessory chromosome is encountered in the second spermatocyte and is so marked that there is no difficulty in tracing its behavior.

6. *What is the behavior of the chromosomes in interkinesis?*

The interval between the two spermatocyte divisions is comparatively brief and the changes in the relative positions of the chromosomes slight. While the chromosomes mass together, the outlines of the individual elements may be seen clearly in favorable preparations of the telophase. Since the whole daughter chromosome complex travels to the pole at about the same rate, its members at the end of the movement lie at approximately the same level. When therefore the second spermatocyte spindle is formed there is little or no change in the positions of the chromosomes, and the equatorial plate of the metaphase corresponds in general with that of the first spermatocyte, from which it was derived. While this is true, the slight movements of all the chromosomes during their poleward migration usually results in a destruction of the typical ring arrangement so characteristic of the first spermatocyte. Because of its isolated position and somewhat greater variation of movement in the anaphases, the accessory shows more marked differences in position in the equatorial plate than the other chromosomes. If it has moved toward the pole at the same rate as the rest of the complex and maintains its position during the telophase it will then be found upon the periphery of the equatorial plate in the second spermatocyte. If, on the contrary, as often happens, the movement is slightly slower than that of the other chromosomes and it swings around under the mass during the anaphase movements it will later appear within the group of chromosomes in the resulting equatorial plate. It is not possible, therefore, to recognize the ac-

cessory in the second spermatocyte by its position, as might frequently be done in the first spermatocyte. I am of the opinion accordingly that any identification based upon position alone is not warranted in the case of the accessory chromosome in the second spermatocyte. By this I do not mean that there may not be similar arrangements of the chromosomes in both spermatocyte metaphases, but rather that the opportunities for movements of the chromosomes during the first spermatocyte anaphase and telophase are so great that no rule may be laid down to mark a type of second spermatocyte. (Plate LXXI, figures 11, 12.)

7. *What is the behavior of the accessory chromosome in the second spermatocyte mitosis?*

At this point in the history of the accessory chromosome of *Anasa* there occurs the wide diversity of opinion between Foot and Strobell and the other investigators who have studied it. The issue is clearly drawn. Foot and Strobell claim that the accessory chromosome is divided in the second spermatocyte as in the first spermatocyte, but Wilson and others are just as positive that it passes undivided into but one of the pair of spermatids formed by the division of each second spermatocyte. Issues are joined here on a question of fact, and it becomes necessary to determine, therefore, whether the accessory chromosome divides in the second spermatocyte metaphase or whether it passes undivided into one of the two daughter cells.

I must confess that it is a matter of no little astonishment to me that there should be any difference of opinion on this point, because the conditions are so clear and unmistakable as almost to preclude error. A number of photomicrographs of the second spermatocyte anaphase have been prepared from both sections and smears and are reproduced in plate LXXI, figures 13-27. In figure 15 there are shown ten pairs of spermatids as they appeared spread upon the glass slip. This slide was prepared according to the method of Foot and Strobell and shows the cells practically separated so that there can be no question of a later division of the accessory chromosome. In each one of these ten pairs it is clearly seen that one member contains an accessory chromosome while its mate lacks it. The same statement holds true of all similar cells drawn and photographed, and of the thousands of others examined. *In no case*

was a divided accessory chromosome found in any second spermatocyte. For a short time the accessory chromosome lies against the mass of chromosomes, but when the spermatids are separated and the nuclei re-form it moves away again and lies at one side. Such conditions are shown in plate LXXI, figures 20-21, 25-27. No clearer or more precise demonstration of a cytological fact could be asked than is here afforded by the unilateral movement of the accessory chromosome.

The question naturally arises, why, if this be true, should two observers decide that the accessory chromosome *does* here divide? It is of course, impossible to explain just why this has occurred, but it would seem to me that Foot and Strobell were impressed with the necessity of showing every chromosome in both daughter cells and that they chose stages which exhibited such arrangements. Since it is impossible to find these conditions after a mid-anaphase, owing to the massing together of the chromosomes, all their pictures were made in the early anaphase while the accessory chromosome lies in or near the equatorial plate. *Not a single photograph of an unmistakably completely divided second spermatocyte is shown among all their figures.* Since it is only at this time that the accessory chromosome may definitely be demonstrated to occur in only one daughter cell their failure to figure it may be understood.

Since Foot and Strobell base their case upon the evidence afforded by their photographs, it is proper that these should be considered. They show in plate III twenty-three figures of second spermatocyte anaphases and telophases. In all but figure 45 the accessory chromosome lies undivided in the equatorial plate, and in this two independent nuclei with no clearly marked accessory chromosomes are advanced as evidence that the accessory chromosome has divided. Figure 46 shows also two nuclei with no indication that they are daughter derivatives. Figures 29, 30, 31 and 44 are introduced to show the actual division of the accessory chromosome. Figure 29 shows the accessory chromosome of a mid-anaphase with a slight constriction at the center, figure 30 a similar cell with two distorted and ill-defined chromosome masses, figure 31 two other similar daughter groups with an apparently divided lagging chromosome, while figure 44 exhibits a mid-anaphase with the daughter (?) groups widely separated and turned by spread-

ing and a broken accessory chromosome midway between them. In all of these cells the chromosomes are thin and of indefinite outline and have apparently suffered much in the process of preparation. There is not a clear case of a divided accessory chromosome in the series, although individual cells, doubtless the best that could be found, are selected and photographed. That later stages show conclusively the undivided accessory chromosome, not alone in selected cases but in every cell in a microscopical field, is demonstrated in plate LXXI, figure 15, of this paper.

The objection may be raised that such a stage does not show all the chromosomes; but it is not a valid objection because it is impossible to photograph all the chromosomes at this stage on account of their apparent fusion into a mass. Moreover the essential facts are admitted by all. It is agreed that all the chromosomes, with the exception of the accessory chromosome, divide so that each group contains ten ordinary chromosomes; it is agreed that the lagging chromosome is the accessory chromosome. The question therefore to be answered concerns the behavior of the lagging chromosome. Are its derivatives found in each of the separated daughter cells of the second spermatocyte; or is it, undivided, included in only one of the two? The evidence on both sides has been presented in statement, drawing, and photograph and the judgment of those interested must, in the absence of personal study, be based upon the presentations. The crux of the situation lies here, for if the accessory chromosome divides there is no dimorphism of the spermatozoa and no visible sexual differentiation of the paternal germ cells, but if it remains undivided then there is a dimorphism of the spermatozoa and a consequent visible chromosome differential between two numerically equivalent classes.

It is pertinent to consider also whether in evaluating the evidence in this case, any considerable number of genuine instances of unusual conditions can be held to invalidate the common conclusions reached by numerous independent students of these phenomena. There can be no question of the occurrence of abnormal conditions in the development of the germ cells. Entire cysts, or even testes, become degenerate from imperfect coördination in development; certain daughter

groups of cells fail to separate and thus produce spermatids with two or four tails and as many simplex groups of chromosomes; minor aberrations occur throughout all stages. Can these be held to prove that there is no constancy in the processes and results of spermatogenesis? Can deviations from the normal or average be regarded as an argument against a type? If so there can be no general laws in biology.